



IsoBind Plant RNA Extraction Kit

Cat No. IB-PRNA-100

System: Silica spin columns (manual workflow)

Sample types: Leafy tissues, woody and fibrous tissue, herbaceous tissue, seed grains, and fruits

USER MANUAL

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1. KIT CONTENTS

The IsoBind Plant RNA Extraction Kit is meticulously designed to provide researchers with a streamlined, efficient tool for extracting high-quality RNA from plant tissues. This kit emphasizes simplicity and reliability, enabling users to achieve optimal RNA yields and purity for a wide array of applications including RT-PCR and other downstream molecular biology processes. This kit is optimized for a variety of plant samples, accommodating the complexities of plant RNA extraction, including the presence of polysaccharides and phenolic compounds.

Component	Description/ Function	Volume Per Sample	Short Term Storage	Long Term Storage	Total for 100 Samples
Lysis Buffer	Lyses cells to release RNA. Specially formulated to ensure complete lysis of cellular material for optimal yield.	600 μ L	2-8°C	Room temperature	60 mL
Binding Buffer *Add Isopropanol (95%) prior to use	Facilitates binding of RNA to the silica membrane.	500 μ L	Room temperature	4-8°C	50 mL
Wash Buffer A *Add EtOH (96-100%) prior to use	Removes impurities such as cellular debris and proteins without stripping away the bound RNA	700 μ L	Room temperature	Room temperature	70 mL
Wash Buffer B *Add EtOH (96-100%) prior to use	Removes residual salts without stripping away the bound RNA	700 μ L	Room temperature	Room temperature	70 mL
Elution Buffer	Elutes purified RNA from the column	50 μ L	Room temperature	4-8°C	5 mL

Silica Spin Columns	Silica matrix that selectively binds RNA while allowing other compounds to pass through it	1 column per sample + collection tube	Room temperature	Sealed in ziplock at 4-8°C	100 units
Proteinase K	Enhances lysis by breaking down proteins, facilitating more efficient RNA release.	20 µL	Room temperature	-20°C	2mL



Buffers contain skin irritants



Wear gloves

2. IMPORTANT NOTES

Before beginning your work with the Gene Vantage IsoBind Plant RNA Kits, please take a moment to review these important notes. Adhering to these guidelines will ensure optimal results and efficiency throughout your extraction process.

Sample Preparation: Achieving a homogeneous sample is crucial for consistent RNA yields. Particularly with complex tissues or plant materials, thorough mechanical breakdown is necessary to ensure all cells are lysed and RNA is accessible. Use a bead mill or tissue homogeniser for solid tissues such as leaf samples and ensure complete mixing with the lysis buffer.

Buffer Preparation: Buffer Inspection and Treatment: Prior to use, inspect all buffers for precipitation which can occur due to cold storage or prolonged shelf life. If precipitates are observed, gently warm the buffers to 37°C, stirring until the solids have dissolved. Cool the buffers to room temperature before application to prevent thermal degradation of RNA.

Centrifugation Parameters: Optimal Speed and Time: Follow the kit's specified centrifugation speeds and times rigorously. These parameters are optimised to ensure maximum recovery of RNA while effectively separating it from proteins, lipids, and other cellular debris. Deviations might lead to lower yields or contamination of the eluted RNA.

Maximum Capacity: To prevent column clogging and ensure efficient RNA purification, do not exceed the recommended sample volume and loading capacity of the spin columns. Overloading can lead to incomplete binding of RNA to the column or carryover of impurities.

Component Stability: Proper storage of kit components is critical for maintaining their efficacy. Store enzymes and sensitive reagents at temperatures specified in the kit documentation to preserve their activity and shelf life. Most reagents in this kit are stable at room temperature, but always check the label for specific storage instructions.

Concentration and Yield: The elution volume can be adjusted based on the desired concentration. A smaller volume results in higher concentration but may reduce overall yield. It's important to balance these factors based on the requirements of subsequent applications.

Optimal Recovery: For optimal recovery, ensure that the elution buffer is in direct contact with the entire surface of the silica membrane by allowing it to incubate on the bench for 2 minutes before centrifuging during the elution step.

Technical Support: Gene Vantage offers comprehensive technical support. If you encounter any issues or have questions about the kit's usage, do not hesitate to contact our technical support team. We are here to help you achieve the best possible results with our products.

3. SAFETY PRECAUTIONS

Ensure the safety of all laboratory personnel by adhering to standard laboratory practices when using the IsoBind Plant RNA kit.

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. Guanidine salts can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Many of the reagents included in the kit are chemical in nature and should be handled in a well-ventilated area. Users should be familiar with the safety data sheets (SDS) for each chemical component for information on potential hazards and first aid measures in case of accidental exposure.

Treat all samples as potentially infectious material. Following the universal precautions for handling biological materials will help protect not only the individual conducting the experiment but also the wider laboratory environment.

Dispose of all waste materials according to your institution's safety guidelines and regulations. This includes the proper disposal of used reagents, consumables, and biological waste to mitigate any potential hazards.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

4. KIT PRINCIPLES

Basic Principle

The IsoBind Plant RNA Kit, designed for efficient plant RNA extraction. Designed with simplicity and reliability in mind, this kit utilizes a single spin column method, ensuring straightforward processing of plant samples while delivering high-quality RNA yields. With the capacity to process up to 100 samples per kit, laboratories can effectively analyze plant gene expression and regulatory mechanisms with ease. Trusted by researchers for its consistent performance, the IsoBind Plant RNA Kit provides a reliable solution for plant molecular biology studies, enabling precise and reproducible RNA isolation. The IsoBind Plant RNA kit provides a versatile solution for a wide range of molecular biology applications, including cloning, sequencing, and gene expression studies. Streamline your research workflows and unlock valuable insights into plant biology with the IsoBind Plant RNA Kit.

The Gene Vantage Plant RNA Extraction Kit operates on the principles of cell lysis, RNA binding, washing, and elution to ensure the efficient extraction of high-quality RNA from plant samples. The process involves:

Tissue homogenization: a crucial step in plant RNA extraction, as it ensures the complete disruption of cell walls and membranes, allowing for the release of RNA. For fresh or frozen plant tissue samples, the tissue is typically ground to a fine powder using a mortar and pestle with liquid nitrogen. The rapid freezing of the tissue by liquid nitrogen makes the cells brittle, facilitating their breakage during grinding. It's important to keep the sample cold during this process to prevent RNA degradation. Once the tissue is homogenized, it can be processed further with a lysis buffer to extract RNA.

Cell Lysis: The first crucial step involves the breakdown of cell membranes to release RNA into the solution. Effective lysis is key to ensuring that all RNA is accessible for subsequent binding. This kit uses Lysis Buffer, which contains a combination of surfactants and a buffering agent that disrupts cellular and nuclear membranes. The addition of Proteinase K, an enzyme, aids in digesting proteins that could otherwise bind nucleic acids and interfere with the extraction process. This enzymatic treatment further ensures that nucleic acids are fully liberated from the cellular membrane. The lysis reaction is enhanced by incubating the sample mixture at 56°C which optimises the activity of Proteinase K and ensures complete lysis.

DNA Binding: Following lysis, the free RNA must be selectively captured or bound while other cellular debris and impurities are excluded. RNA in the lysate binds to a silica membrane within the spin column when in the presence the Binding Buffer. This environment promotes the adherence of nucleic acid to the silica surface due to the formation of hydrogen bonds between the negatively charged phosphate groups of the nucleic acids and the positively charged surface of silica. This step is critical as it determines the yield of nucleic acid.

Washing: Clean RNA is essential for sensitive applications like PCR. The kit contains two sequential wash buffers (Wash A and B), each designed to efficiently remove different types of contaminants. Wash Buffer A primarily removes proteins and other large organic molecules, Wash Buffer B is designed to eliminate smaller molecules and salts. Each wash involves adding a specific volume of buffer, followed by centrifugation to pull the liquid through the column while the nucleic acid remains bound to the silica membrane. This ensures that only purified nucleic acid remains on the column.

Elution: The final step is to release the purified RNA from the silica membrane for use in downstream applications. Elution is achieved by applying an Elution Buffer, which disrupts the hydrogen bonds between the nucleic acid and the silica, allowing the nucleic to be released into the buffer. The elution buffer is pre-warmed to enhance the efficiency of nucleic acid recovery. The nucleic acid is collected by centrifugation, which forces the eluted RNA into a clean microcentrifuge tube.

Key Features:

Quality of Output: Utilises advanced silica-based spin column technology, which selectively binds RNA while efficiently removing contaminants. This results in RNA with high purity, characterised by optimal A260/A280 ratios typically ranging between 1.8 and 2.0, indicating minimal protein contamination and readiness for sensitive downstream applications.

Comprehensive Cell Disruption: The Lysis Buffer and Proteinase K combination effectively disrupts a wide variety of cell types, ensuring complete release of RNA.

Enhanced Recovery: Tailored for samples that are difficult to lyse, the system ensures that even tightly bound nucleic acids are made available for extraction, which is critical for achieving consistent results across different sample types.

Time Efficiency: The entire RNA extraction process can be completed in approximately 45 minutes for 24 samples, which is ideal for labs seeking to maintain their turn around times without compromising on the quality of results.

Ease of Use: The protocol is designed to be straightforward with clear step-by-step instructions, reducing the potential for operator error and the need for extensive training.

Streamlined Approach: Specifically optimised for the extraction of viral RNA from a variety of agricultural and human samples, the kits robust lysis and binding conditions are effective in isolating high quality RNA.

Compatibility with Downstream Applications: The high-quality RNA extracted is suitable for a variety of molecular biology techniques, including PCR, qPCR and next-generation sequencing, ensuring broad applicability.

Scalability: The kit is suitable for both low and high-volume sample processing, with options for manual (individual spin column) and semi-automated (96 well spin plates) workflows. This flexibility allows laboratories of all sizes to integrate this kit into their existing workflows efficiently.

Note: Please engage with **Gene Vantage** technical support (see above: Important Notes) should you require a higher throughput

5. HARDWARE AND CONSUMABLES (SUPPLIED BY THE USER)

5.1 Hardware

Centrifuge:

A high-speed centrifuge capable of achieving at least 13,000 x g is essential for the effective sedimentation of cellular debris and the precise separation of supernatants during the RNA extraction process.

The centrifuge must be reliable and capable of maintaining consistent speeds to avoid variations that could affect the purity and yield of the extracted RNA. A temperature control feature to protect sensitive samples from heat degradation during extended spin cycles.

Vortex Mixer:

A vortex mixer is required to thoroughly mix samples with lysis and binding buffers, which is crucial for the complete lysis of cells and the homogeneous suspension of RNA within the solution. This ensures maximum contact between the RNA and the silica binding surface, increasing the efficiency of RNA recovery.

Thermomixer/ heating block/ oven:

Required for the incubation of samples at controlled temperatures during the lysis and elution steps. The ability to set precise temperatures is essential, as optimal lysis conditions can vary depending on the sample type and the specific requirements of the RNA extraction protocol.

5.2 Consumables

Microcentrifuge Tubes (1.5 mL)

Used for sample preparation and for collecting the eluted RNA.

Pipettes and Aerosol-Barrier Pipette Tips:

Precision pipettes and aerosol-barrier tips are crucial for the accurate measurement and transfer of fluids, which is vital for maintaining the correct buffer ratios and avoiding cross-contamination

between samples. This is particularly important when working with infectious agents or when performing multiple extractions to ensure reproducible and reliable results.

The pipettes should be regularly calibrated to ensure accuracy, and the tips should be certified RNase-free to prevent the degradation of RNA by residual enzymatic activity.

Ethanol (96-100%, molecular grade)

Added to wash buffers to help in washing away impurities without stripping the RNA from the column.

Isopropanol (95%, molecular grade)

Added to binding buffer to improve the yield and quality of RNA by ensuring more efficient binding of RNA to the column.

DNase:

Deoxyribonuclease (DNase) is used to remove DNA contamination from RNA samples, ensuring that the extracted RNA is pure and suitable for downstream applications. This is an optional step that can be included at the users discretion should the downstream assay require it (e.g cDNA synthesis)

6. QUICK VIEW PROTOCOL

Step	Procedure	Details
Sample Preparation	Mechanical Disruption	Use a mechanical homogenizer to ensure uniform disruption of plant tissues, especially tough materials like stems or roots. Operate at high speed for 1-2 minutes until the tissue is finely powdered. This method achieves maximum cell disruption, crucial for releasing all cellular contents, including RNA.
Lysis	Lysis Buffer Addition	Combine the powdered tissue with lysis buffer, ensuring the addition of beta-mercaptoethanol to a final concentration of 1%. Incubate the mixture at 56°C for 10 minutes. Vortex the sample briefly after adding the lysis buffer to ensure thorough mixing and complete coverage of the plant material. This step enhances the efficiency of cell lysis and RNA release.

RNA Binding	Binding Buffer Addition and Centrifugation	After lysis, add an equal volume of binding buffer to the lysate. Transfer the mixture to a spin column placed in a collection tube. Centrifuge at 10,000 x g for 1 minute. This process ensures that RNA binds selectively to the silica membrane while contaminants are washed through.
Washing	Sequential Wash Buffer Applications	Apply 700 µL of Wash Buffer A to the spin column, then centrifuge at 10,000 x g for 1 minute to remove most soluble impurities. Repeat the process with 700 µL of Wash Buffer B to ensure all remaining contaminants are removed. These steps are critical for achieving high-purity RNA, essential for sensitive downstream applications.
RNA Elution	Elution Buffer Application and Centrifugation	Add 50-100 µL of elution buffer directly onto the silica membrane. Allow it to stand for 1 minute to fully hydrate the membrane, improving elution efficiency. Then centrifuge the column at 10,000 x g for 1 minute to collect the eluted RNA in the collection tube. The RNA is now ready for immediate use or can be stored at -80°C for long-term preservation.

7. KIT SPECIFICATIONS

Parameter	Specification
Format	Spin columns
Sample Material	Plant tissue
Typical Yield	Varies with sample type
Purity Ratios	A260/A280 > 1.8
Elution Volume	30-100 µL
Preparation Time	Approximately 60 minutes
Binding Capacity	Up to 100 µg of RNA

8. WORKFLOW TIPS

To maximize the effectiveness and reliability of the IsoBind Plant RNA Kit, it is crucial to consider additional aspects of the extraction process that impact both the quality of the RNA obtained and the user's experience. These additional suggestions provide guidance on sample quality and preparation, elution efficiency, and quality control measures:

COLLECTION AND STORAGE OF STARTING MATERIAL

Immediate Processing: Ideally, samples should be processed immediately after collection to minimise RNA degradation. If immediate processing is not possible, samples must be handled and stored carefully to preserve their integrity.

Plant Tissues: Plant tissues are particularly susceptible to enzymatic degradation because of their high RNase content. To ensure the integrity of RNA, clean and segment the plant material quickly after harvesting. Freeze plant segments in liquid nitrogen immediately after cleaning and cutting. Store the frozen samples at -80°C in RNase-free containers until ready for RNA extraction. Avoid thawing during transfer to preserve RNA quality.

Storage Considerations: Long-term storage conditions play a crucial role in maintaining RNA integrity. Samples stored at -80°C are generally stable for several years. Avoid frequent temperature fluctuations, as these can lead to ice crystal formation and mechanical breakdown of cellular structures, facilitating RNase activity. For short-term storage (a few days to weeks), refrigerating samples at -20°C may be adequate, but is not recommended for samples sensitive to partial degradation.

Sample Size Considerations

- **Optimal Sample Size:** Use a consistent sample size (typically around 100 mg) for reproducible RNA yield and quality. This standardization helps in optimizing the buffer volumes used and the efficiency of the extraction process.
- **Scaling Buffer Volumes:** If deviating from the standard 100 mg tissue sample, adjust the volumes of lysis, binding, wash, and elution buffers proportionally. For example, increasing the sample size to 200 mg would require doubling the volume of all buffers to maintain the buffer-to-sample ratio crucial for effective RNA isolation.

9. PREPARING BUFFERS AND EQUIPMENT

Before Starting:

Centrifuges

Performance Check: Before beginning any procedures, ensure that the centrifuge is functioning correctly. Perform a test run to check for any unusual noises or vibrations that could indicate a maintenance issue. Ensure that the rotor is securely fastened and that the lid closes properly.

Calibration: Regular calibration of the centrifuge is crucial for achieving the precise speeds necessary for optimal RNA isolation. Inaccuracies in speed can lead to inefficient separation of phases, potentially contaminating the RNA sample or resulting in lower yields.

Cleaning: Clean the centrifuge and rotor regularly to prevent the buildup of dust and biological material, which could interfere with operations or contaminate samples. Use appropriate

disinfectants to wipe down the interior and rotor, especially after handling potentially infectious samples.

Pipettes

Accuracy Verification: Verify the accuracy of all pipettes before use. This can be done by pipetting distilled water onto a precision scale to check if the dispensed volumes are within the manufacturer's specified tolerance.

Calibration: Calibrate pipettes regularly according to the manufacturer's guidelines to ensure they dispense volumes accurately, which is critical for the precise preparation of buffers and reagents.

Maintenance: Clean pipettes frequently to prevent cross-contamination between samples. Check the pipette tips for any residual sample before each use, and replace pipette tips between samples to maintain sample integrity.

Vortex Mixer

Functionality Check: Ensure that the vortex mixer is operating correctly. Test the mixer by running it at different speeds to ensure it can provide the vigorous agitation needed for thorough mixing of lysis buffers with samples.

Stability: Check the stability of the vortex mixer on the bench to prevent any movement during operation, which could affect the homogeneity of sample mixing.

Balances

Calibration and Accuracy: Regularly check and calibrate balances used to weigh samples or reagents to ensure precision. Incorrect measurements can alter the concentration of reagents, affecting the efficiency of the RNA extraction.

Cleanliness: Keep the balance area clean and free from vibrations and drafts, which could affect the accuracy of measurements.

Preparation: Prepare all consumables in advance by arranging them in an orderly manner on the workstation. This organization helps prevent confusion and potential contamination during the extraction process.

Ensure that all reagents are within their expiration dates and have been stored under the correct conditions. Any reagent that appears cloudy or precipitated should be warmed gently, if permissible, and mixed thoroughly to redissolve any solids.

Workspace Preparation: Disinfect the workspace thoroughly before starting the extraction to create an RNase-free environment. Use RNase decontamination solutions and maintain clean bench practices throughout the procedure.

10. COMPLETE PROTOCOL

1. Sample Collection and Preparation

1.1 Tissue Homogenization: Fresh or frozen plant tissue (up to 100 mg) should be quickly frozen in liquid nitrogen and ground to a fine powder using a mortar and pestle. This process ensures that cellular structures are thoroughly disrupted, allowing for optimal RNA release.

Precautions: Maintain samples and tools at low temperatures to prevent RNA degradation.
Optional: Use a tissue lyser for difficult to extract samples. This helps to fully disrupt the sample and ensure that nucleic acids are released and available for the extraction process.

2. Lysis

2.1 Adding Lysis Buffer: Transfer the ground tissue to a microcentrifuge tube and add 600 μ L of Lysis Buffer pre-mixed with beta mercaptoethanol. Vortex the mixture vigorously for 15 seconds to ensure thorough mixing and complete lysis of cells.

2.2 Incubation: Allow the lysate to sit at room temperature for 5 minutes to ensure complete breakdown of cell components, facilitating maximum RNA release.

3. RNA Binding

3.1 Ethanol Addition: Add 600 μ L of 96-100% ethanol to the lysate and mix well by pipetting. This step prepares the RNA molecules for binding to the silica membrane in the spin column.

3.2 Column Loading: Transfer the lysate-ethanol mixture to the spin column placed in a collection tube.

3.3 Centrifuge at 16,000 x g for 1 minute to bind RNA to the column.

4. Washing

4.1 First Wash: Add 700 μ L of Wash Buffer A to the column.

4.2 Centrifuge for 1 minute at 16,000 x g and discard the flow-through.

4.3 Second Wash: Add 700 μ L of Wash Buffer B, repeat centrifugation, and discard the flow-through.

4.4 Drying the Membrane: After the final wash, centrifuge the column for 2 minutes at 16,000 x g to completely dry the membrane. This step is crucial to remove any remaining ethanol, which could interfere with downstream applications.

5. RNA Elution

5.1 Transfer the spin column to a clean 1.5 mL microcentrifuge tube.

5.2 Add 50-100 μ L of Elution Buffer directly to the center of the spin column membrane.

5.3 Let it sit for 1 minute at room temperature to allow RNA to dissociate from the membrane.

5.4 Centrifuge at 16,000 x g for 1 minute to collect the eluted RNA.

11. TROUBLESHOOTING GUIDE

Problem Description	Possible Causes	Suggestions
Low RNA yield	Incomplete lysis of sample tissue	Ensure thorough homogenization of the sample. Increase the amount of Lysis Buffer if necessary.
	Insufficient incubation time	Extend the incubation time during the lysis step.
	RNA degradation	Use fresh samples or store samples properly. Add RNase inhibitors if needed.
Poor RNA purity (low A260/A280 ratio)	Contamination with proteins or phenolic compounds	Increase the number of wash steps. Ensure complete removal of Wash Buffers.
	Incomplete removal of wash buffers	Perform additional dry centrifugation steps to remove residual wash buffers.
RNA degradation	Improper sample storage	Store samples at -80°C or use immediately after collection.
	Prolonged exposure to room temperature	Keep samples and lysates on ice during the extraction process.
Inconsistent RNA yield	Variation in sample size or type	Standardize the amount and type of starting material.
	Inconsistent execution of the protocol	Follow the protocol steps precisely and consistently.
Clogged spin column	Excessive sample material	Reduce the amount of starting material or increase the volume of Lysis Buffer.
	Insufficient centrifugation	Increase the centrifugation speed or duration.
Contamination in RNA samples	Cross-contamination between samples	Use sterile equipment and consumables. Practice good laboratory hygiene.
	Contamination of reagents or equipment	Use fresh reagents and clean equipment before use.
Equipment malfunction	Centrifuge not reaching required speed	Check the centrifuge settings and performance. Calibrate or repair the centrifuge if necessary.

	Pipettes delivering inaccurate volumes	Calibrate pipettes regularly. Use pipettes with the correct volume range for the protocol.
Buffer Precipitation	Cold storage of buffers that should be at room temperature	Ensure all buffers are stored according to the specifications provided in the manual. Label storage containers clearly with the appropriate storage temperatures and routinely check storage conditions.
	Incorrect preparation of buffers	Adhere strictly to the buffer preparation instructions provided in the manual. Measure all components accurately and mix thoroughly
Difficulty in Eluting RNA	Spin Column membrane dried out	Pre wet the column before adding the elution buffer with a few micro litres of RNase free water. Ensure the spin column does not sit out for too long after the final wash step.
	Elution buffer not adequately heated.	Heat the elution buffer to the specified temperature. Let it incubate in the column before centrifugation. If yield is still low, perform a second elution

12. PRODUCT USE RESTRICTION / WARRANTY

GENE VANTAGE kit components are intended, developed, designed, and sold for research purposes only. All kit components are for general laboratory use only and should only be used by qualified personnel wearing the appropriate protective clothing. GENE VANTAGE does not assume any responsibility for damages due to improper application of our products in other fields of application. Any user, whether by direct or resale of the product, is liable for any and all damages resulting from any application outside of research.

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